Visualising Mechanoelectrical Transduction in Cancers

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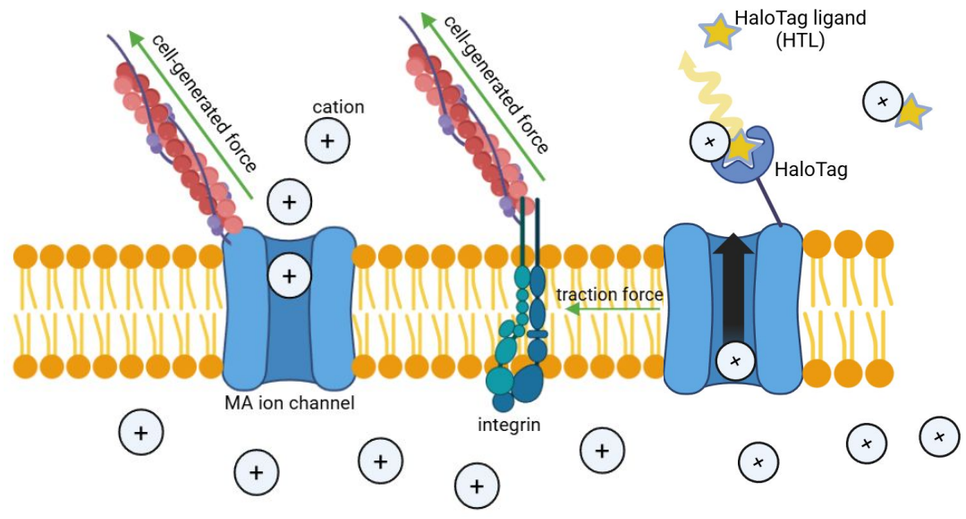
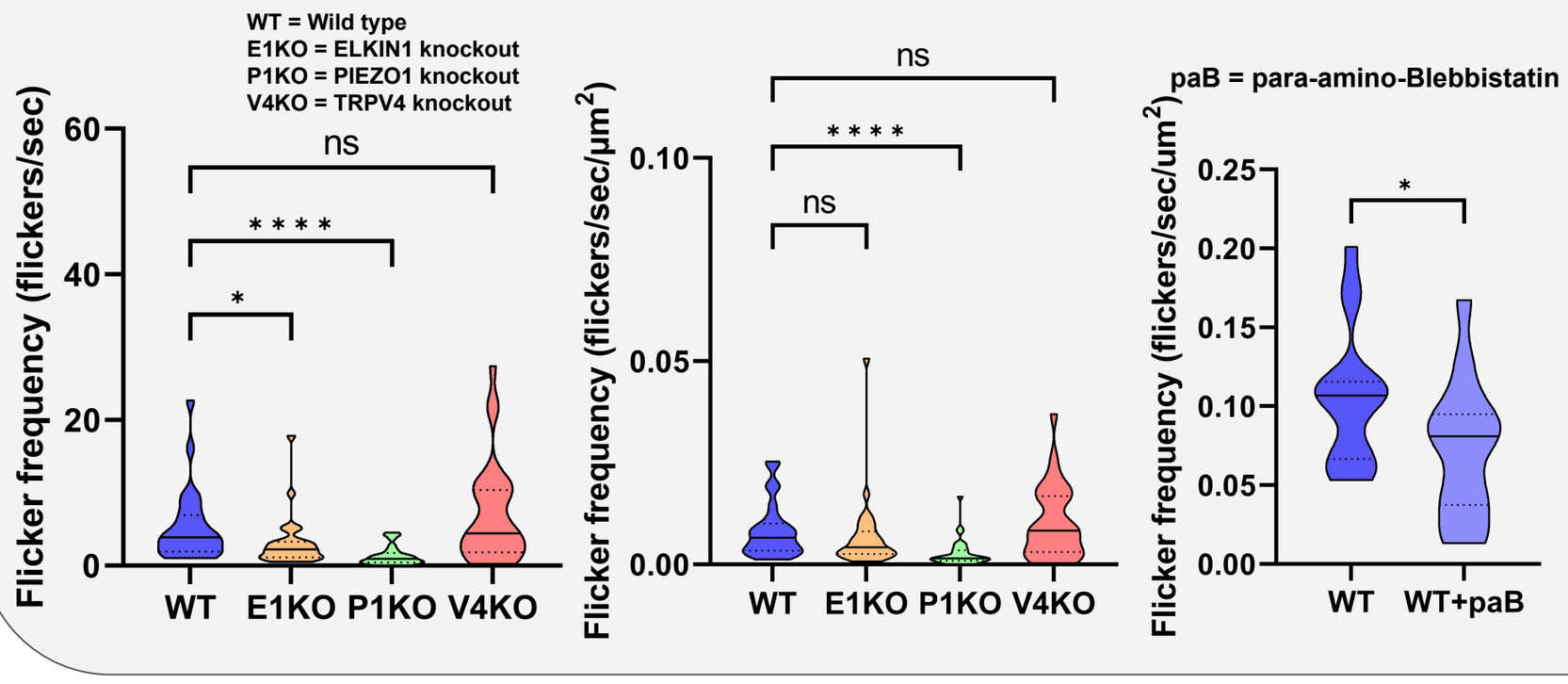
Cancer cell lines are known for being highly mechanically responsive, and often express multiple different mechanically-activated (MA) ion channels. However, it is not well understood why cancers possess so many MA channels and if their pattern of signalling differs within the cell-substrate interface. We have previously used TIRF microscopy combined with cell-wide Ca^{2+} dyes to visualise these channels activating via cell-generated forces (i.e. mediating cell-intrinsic signalling) in the melanoma WM266 cell line, and were able to determine where two individual channels were activating. However, global Ca^{2+} dyes are less effective in cell lines with vastly diverse channel activity, i.e. they lack the specificity required.

Here, we have identified the self-labelling protein tag, HaloTag, as a potential tool to visualise the cell-intrinsic signalling of specific channels in the MCF7 breast cancer cell line.



Ca^{2+} flickers in the MCF7 breast cancer cell line - predictable or complex?

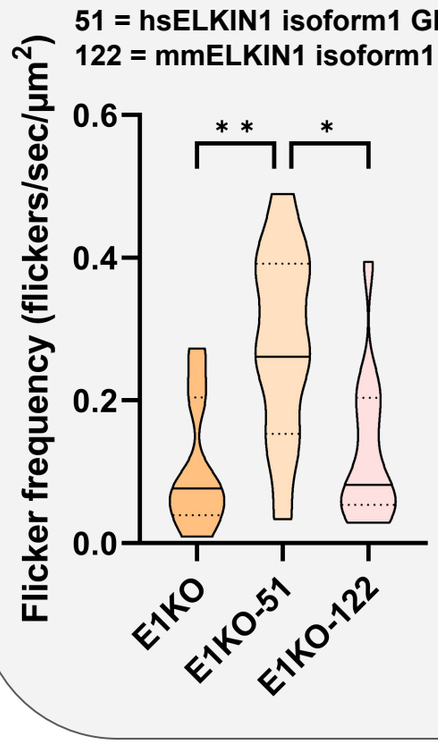


paB is an inhibitor of actomyosin contractility.

- Subset of Ca^{2+} flickers are potentially dependent on ELKIN1 in MCF7 cells.
- Suggests that PIEZO1 is also mediating spontaneous flickers.
- No indication that TRPV4 is responsible for flickers, although its nature as a polymodal channel would suggest that a reduction should be seen.
- Some flickers are dependent on cell-generated forces but this effect is not as robust as in other cell lines.

The MCF7 cell line poses a complex Ca^{2+} environment. This data highlights the limitations in using channel knockouts and global Ca^{2+} indicators.

Identifying candidates for direct tagging

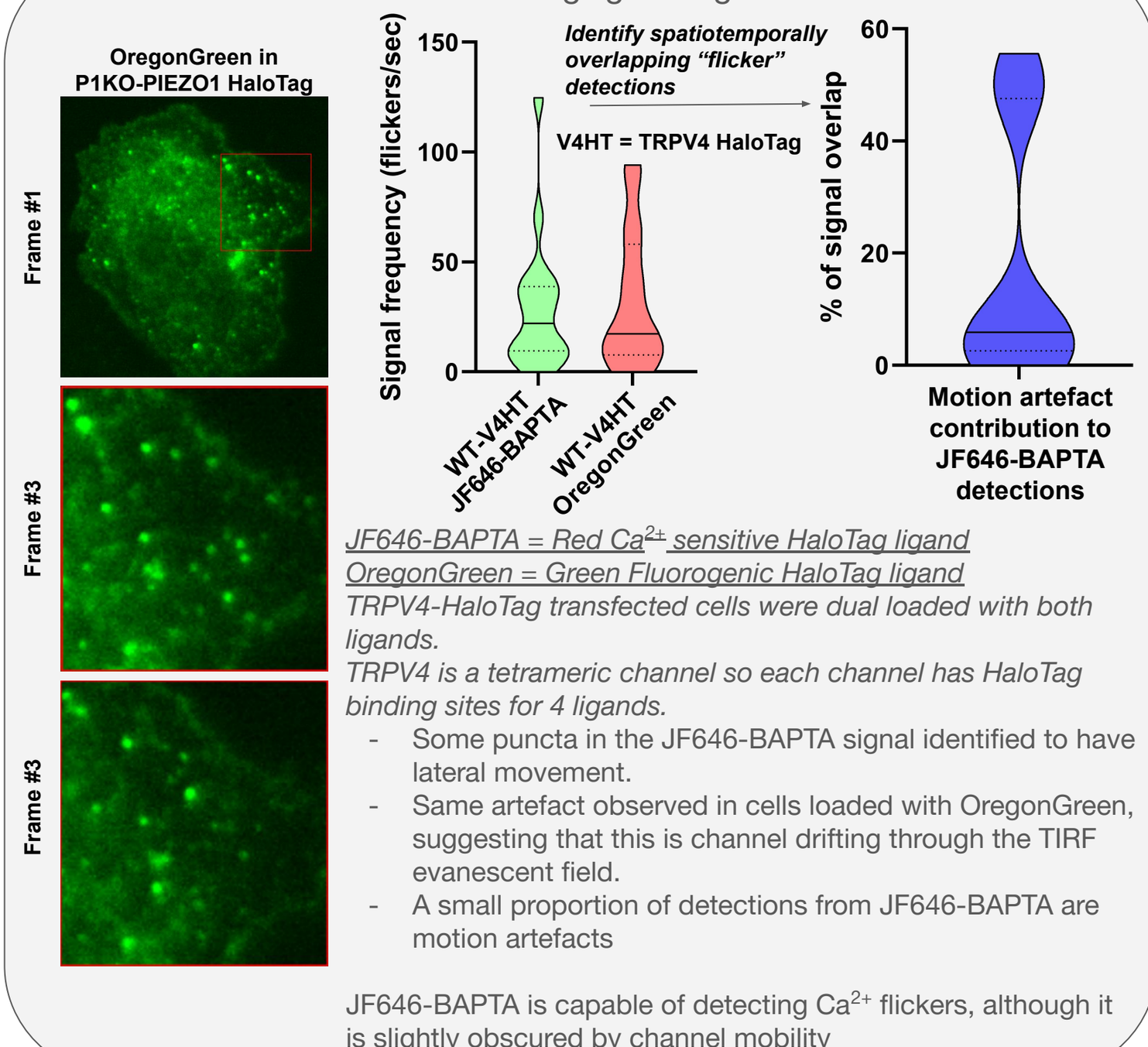


Human and mouse ELKIN1 are highly conserved.

- Overexpression of human ELKIN1 in E1KO cells restores flickers.
- Overexpression of mouse ELKIN1 does not restore flickers.

A subset of flickers in MCF7 cells are likely dependent on the presence of ELKIN1. The interaction between ELKIN1 and other channels is likely not the reason for the observed increase of flickers

What does the HaloTag ligand signal look like?

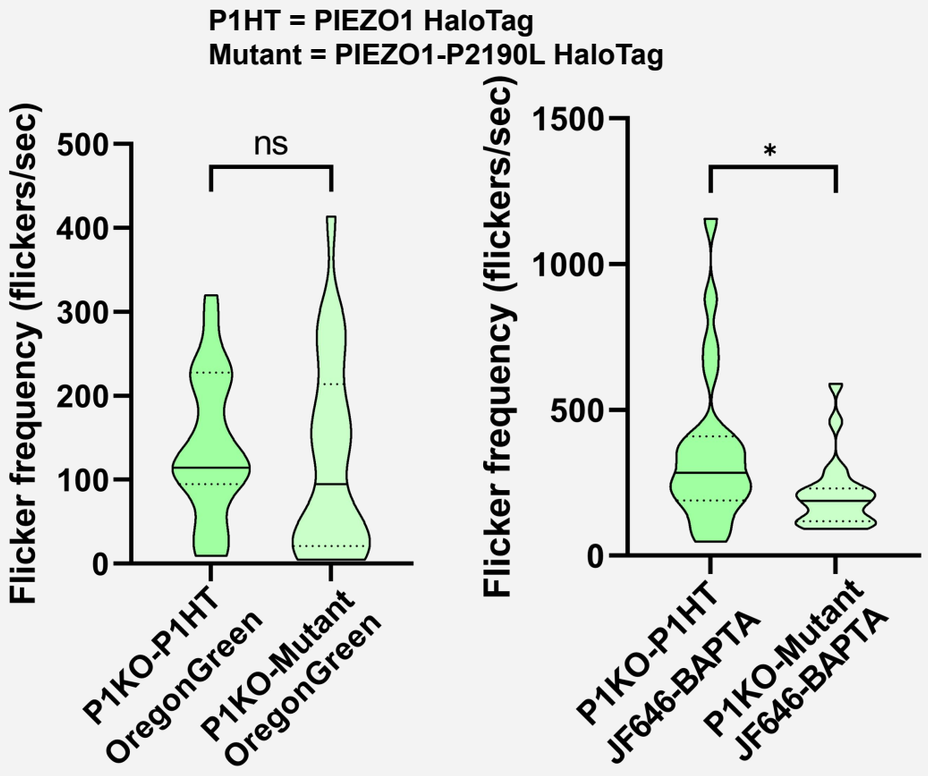


JF646-BAPTA = Red Ca^{2+} sensitive HaloTag ligand
OregonGreen = Green Fluorogenic HaloTag ligand
TRPV4-HaloTag transfected cells were dual loaded with both ligands.
TRPV4 is a tetrameric channel so each channel has HaloTag binding sites for 4 ligands.

- Some puncta in the JF646-BAPTA signal identified to have lateral movement.
- Same artefact observed in cells loaded with OregonGreen, suggesting that this is channel drifting through the TIRF evanescent field.
- A small proportion of detections from JF646-BAPTA are motion artefacts

JF646-BAPTA is capable of detecting Ca^{2+} flickers, although it is slightly obscured by channel mobility

Does the HaloTag tool offer sufficient specificity?

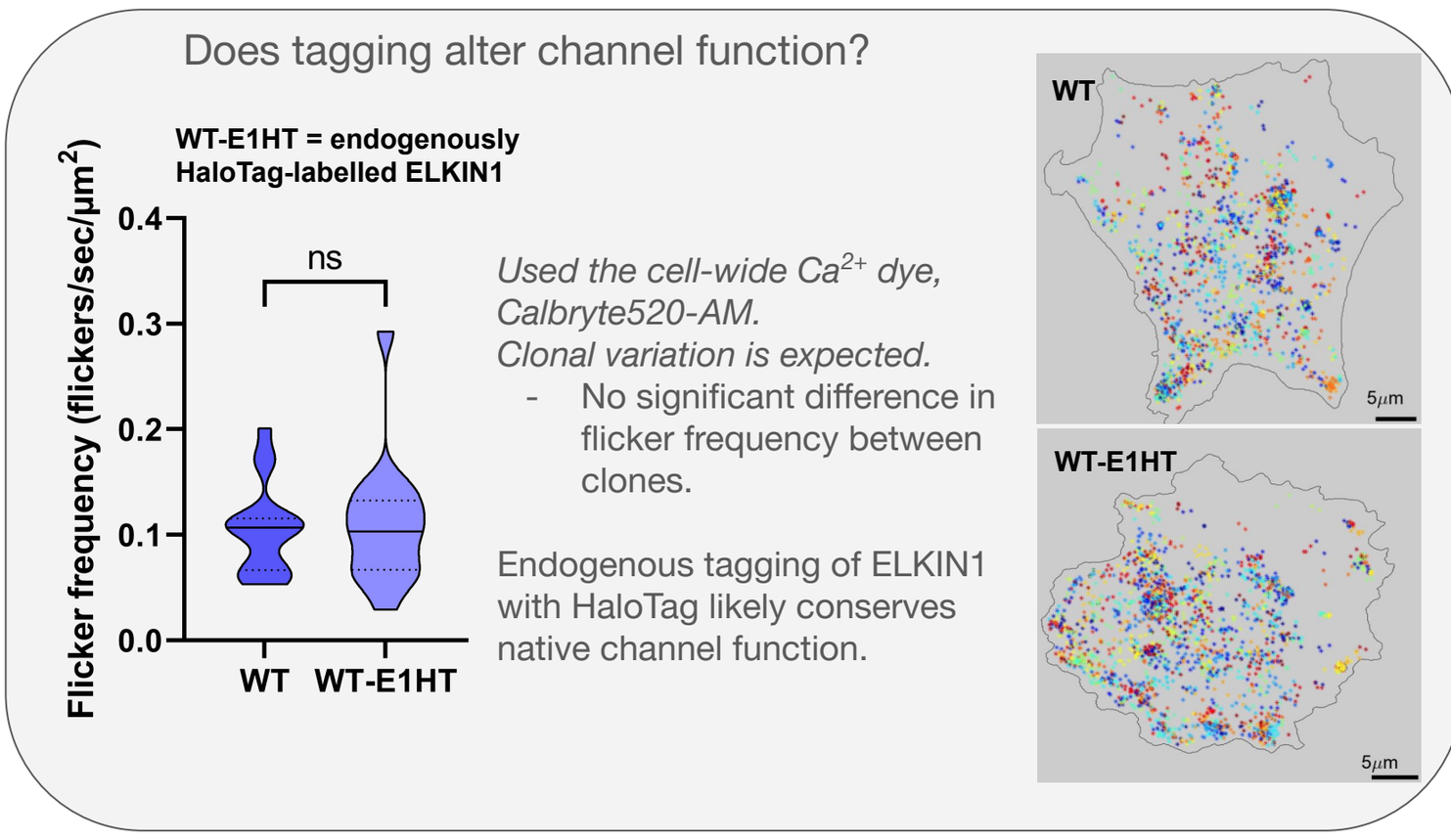


The PIEZO1 P2190L mutation results in a channel that traffics to the membrane but cannot open. This is our negative control.

- No significant difference in "flickers" between variants when using OregonGreen. Essentially looking at channel expression.
- Fewer flickers in mutant PIEZO1-HaloTag compared to wildtype PIEZO1-HaloTag when using Ca^{2+} sensitive ligand. The ligand isn't only detecting motion artefacts, otherwise there would be no difference between variants.

The difference in signal between variants loaded with JF646-BAPTA indicates that the ligand is specifically detecting Ca^{2+} influx through the labelled channel.

Does tagging alter channel function?



Used the cell-wide Ca^{2+} dye, Calbryte520-AM.
Clonal variation is expected.

- No significant difference in flicker frequency between clones.

Endogenous tagging of ELKIN1 with HaloTag likely conserves native channel function.

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